Biocatalysed reduction of carboxylic acids to primary alcohols in aqueous medium: a novel synthetic capability of the zygomycete fungus Syncephalastrum racemosum.

Biocatalysed Reduction of Carboxylic Acids to Primary Alcohols in Aqueous Medium: a Novel Synthetic Capability of the Zygomycete Fungus *Syncephalastrum racemosum*

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Abstract

The zygomycete fungus *Syncephalastrum racemosum* shows the remarkable capability of reducing carboxylic acids to primary alcohols in water medium, at ambient temperature and pressure. The reaction does not require molecular hydrogen, and in most cases affords quantitative transformations. The results herein reported highlight the basic molecular scaffold that can be accepted by the fungus, the effects due to substituents, and also the possibility that carboxylic acids can be generated in the reaction medium by enzymatic hydrolysis of the corresponding methyl esters. This biocatalysed reduction implements the scarcely supplied enzymatic toolbox for the conversion of carboxylic groups into primary alcohols, which can be exploited for the optimization of sustainable synthetic procedures.

1. Introduction

The reduction of esters and carboxylic acids to primary alcohols is a key reaction of organic chemistry,[1] which has widespread application in the manufacturing processes of fine and bulk chemicals. This transformation still relies on the stoichiometric use of metal hydride reagents and their derivatives,[2] often with the requirement of extensive heating, and anhydrous conditions. The highly exothermic hydrolytic work up is generally troublesome, and yields voluminous precipitates. Recently, transition metal catalysed hydrogenation reactions [3] employing molecular hydrogen as a reducing agent have been investigated in order to address the waste challenge connected with hydride promoted reductions. However, only a limited number of these new procedures work at room temperature and low hydrogen pressure. Other chemocatalytic methods, such as the metal-catalyzed hydrosilylation of esters, have received considerable interest as a tool for carboxylic group reduction to primary alcohols.[4] Nevertheless, the development of a cost-effective, and highly selective catalyst for this transformation is still desirable because most of the known procedures either require expensive silanes or have limited functional group tolerance.

As for biocatalysed methods, this is a very challenging task, because the reduction of acids is characterised by a very low redox potential, and it has been described up to now only for a very limited number of microorganisms and cultured plant cells. The two classes of enzymes which have been reported so far as biocatalysts of this reaction are:[5] carboxylic acid reductases (CARs, E.C.1.2.1.30) from aerobic sources, such as bacteria, fungi and plants, and aldehyde ferredoxin oxidoreductases (AORs, E.C. 1.2.99.6) from anaerobic bacteria and archaea.

In 1969 Gross *et al.* isolated a CAR [6] from *Neurospora crassa* and performed preliminary studies on the reaction mechanism. The reduction was found to be adenosine triphosphate (ATP), Mg^{2+} and NADPH dependent.[6c] An homologous enzyme was isolated and purified by Rosazza and co-workers from *Nocardia* species NRRL 5646 in 1997.[7] This CAR enzyme was overexpressed, employed successfully in biocatalytic reductions,[7a,c] and its reaction mechanism was fully elucidated.[7d] Recently,[8] the conversion of 3,4-dihydroxyphenylacetic acid into 3-hydroxytyrosol

has been performed in whole *Escherichia coli* BL21(DE3) cells overexpressing CAR from *Nocardia* and phosphopantetheinyl transferase from *E. coli,* with the concomitant action of an endogenous *E. coli* aldehyde reducing activity. In 2013,[9] Turner *et al.* described the conversion of fatty acids into the corresponding alcohols, by coexpression of a CAR from *Mycobacterium marinum* and an aldehyde reductase from *Synechocystis* species.

Sodium carboxylates C_6-C_{10} were transformed into the corresponding alcohols by growing cultures of the fungus *Colletotrichum gloeosporioides*, but the method lacks general applicability to different substrates.[10] Some examples of aerobic reductions of aromatic carboxylic acids by fungi have been described, especially in connection with the conversion of ferulic acid into vanillin.[5]

Resting cells of *Clostridium thermoaceticum* and *Clostridium formicoaceticum* were described to reduce [11] carboxylic acids and carboxylates to alcohols at the expense of carbon monoxide or, less efficaciously, in the presence of formate. The enzymes responsible for the carboxylic acid reduction in these two organisms were isolated and characterised to be highly oxygen sensitive tungsten enzymes (W-AORs). In spite of the efficiency of the reaction, the requirement for strictly anaerobic conditions makes this alternative poorly appealing for preparative applications.

In 1999, it was first reported [12a] that the hyperthermophilic archaea *Pyrococcus furiosus* reduced carboxylic acids with starch as carbon and energy source at 90°C. Recently, the use of this microorganism has been reconsidered,[12b] because it contains suitable enzymes to couple the oxidation of molecular hydrogen (catalysed by hydrogenases) to the reduction of carboxylic acids to aldehydes (mediated by an AOR). The presence of endogenous alcohol dehydrogenases promotes the final conversion of aldehydes into primary alcohols. A biocatalytic hydrogenation procedure has been optimised for the reduction of carboxylic acids at 40°C under 5 bar molecular hydrogen pressure. The same authors have also described the possibility to employ syngas instead of hydrogen to promote this *P. furiosus*-mediated reduction, thus hypothesising the presence in this microorganism of enzymes capable of coupling CO oxidation to acid reduction.[12c]

As for eukaryotic cells, nine different plant cell cultures have also been successfully employed on several carboxylic acids, but only in modest yields and with long pre-culture times and reaction times.[13]

We now wish to report on the possibility of preparing primary alcohols by reduction of carboxylic acids in a very mild and efficient way, using the whole cells of the zygomycete fungus *Syncephalastrum racemosum* in aqueous medium, at room temperature and pressure. In some cases the carboxylic acids can be generated in the reaction medium by enzymatic hydrolysis of the corresponding methyl esters. This peculiar reducing activity was discovered during a functional screening of the enzymatic capabilities of a selection of filamentous fungi of the *Mycotheca Universitatis Taurinensis* (MUT), performed on model substrates in the aim of finding new biocatalysts for synthetic applications.

2. Experimental

2.1 Materials and equipment

The carboxylic acids employed for the work were commercial products purchased from Sigma-Aldrich. The corresponding methyl and ethyl esters were either commercial products from Sigma-Aldrich or were prepared by Fischer esterification. Stock solutions (500 mM) of each substrate in DMSO were employed for the biotransformations.

GC/MS analyses were performed on an Agilent HP 6890 gascromatograph equipped with a 5973 mass detector and an HP-5-MS column (30 m × 0.25 mm × 0.25 μm, Agilent), employing the following temperature program: 60°C (1 min) / 6°C min^{-1} / 150°C (1 min) / 12°C min^{-1} / 280°C (5 min). The identity of the reduction products was established by comparison with reference samples (either commercially available or prepared by lithium aluminium hydride reduction of the corresponding methyl esters).

2.2 Fungal strains

The strain of the zygomycete fungus *Syncephalastrum racemosum* MUT 2770 employed for this work is preserved at *Mycotheca Universitatis Taurinensis* (MUT, Department of Life Sciences and Systems Biology, University of Turin).

2.3 Biotransformation experiments

Fungal strains were pre-grown in Petri dishes containing malt extract solid medium (MEA: 20 g L^{-1} glucose, 20 g L^{-1} malt extract, 20 g L^{-1} agar, 2 g L^{-1} peptone) from which the inoculum for liquid cultures was set up. The fungus was inoculated as conidia suspension ($1 \cdot 10^6$ conidia/mL) in 50 mL flasks containing 40 mL of malt extract liquid medium. Flasks were incubated at 25°C and were maintained under agitation (110 rpm).

After 2 days of pre-growth, a 500 mM solution of the substrate in DMSO was added, to a starting substrate concentration (c_0) of 1-5 mM. For each substrate, three biological replicates were run. The experiment was run for 3 days after the addition of the substrates, during which time 1 mL samples were taken, at specified intervals (usually 24, 48, and 72 h). Each sample was extracted with EtOAc (500 µL), the organic phase was dried over anhydrous Na_2SO_4 and analysed by means of GC/MS. In some cases (see Section 2.4) the isolation of the reduced product has been carried out.

For each set of biotransformations, one flask was used to measure the initial biomass and pH before the addition of the substrate. These parameters were also evaluated at the end of the experiment for all the flasks. The liquid media was separated from the biomass by filtration and was used for pH measurement while the mycelia were dried at 60°C for 24 h to measure the biomass dry weight.

2.4 Isolation procedure of biotransformation products

In the case of benzoic acid, methyl benzoate, phenylacetic acid, methyl phenylacetate, 3-phenylpropanoic acid, phenoxyacetic acid, methyl phenoxyacetate, 2-furoic acid, 2-(thiophen-2-yl)acetic acid, methyl 2-(thiophen-2-yl)acetate, 2,4-hexadienoic acid, methyl 2,4-hexadienoate, and

methyl octanoate the isolation of the final products was carried out. For benzoic acid, 2-(thiophen-2-yl)acetic acid and 2,4-hexadienoic acid c_0 = 1 mM was employed, while for all the other substrates c_0 = 5 mM was used (in both cases the reaction total volume was 40 mL). After 72 h, the reaction mixture was filtered to remove the biomass. The filtrate was extracted with EtOAc (3 × 20 mL), dried on Na_2SO_4, and concentrated under reduced pressure. When the conversion was not complete (GC/MS analysis) the residue was purified either by treatment with a saturated $NaHCO_3$ solution or by column chromatography (silica gel, elution with hexane and increasing amount of EtOAc).

Benzyl alcohol: from benzoic acid (3.8 mg, 89%) and from methyl benzoate (19.9 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.35-7.25 (m, 5H, aromatic hydrogens), 4.67 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 141.2, 128.3, 127.3, 126.9, 65.0. GC/MS: t_R = 6.46 min, *m/z* 108 (M^+, 95), 91 (20), 79 (100).

1-Phenylethanol: from phenylacetic acid (9.0 mg, 37%) and from methyl phenylacetate (6.3 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.35-7.15 (m, 5H, aromatic hydrogens), 3.85 (t, 2H, *J* = 6.6 Hz, $PhCH_2CH_2OH$), 3.85 (t, 2H, *J* = 6.6 Hz, $PhCH_2CH_2OH$). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 138.4, 129.3, 128.6, 126.5, 63.2, 39.4. GC/MS: t_R = 8.19 min, *m/z* 122 (M^+, 27), 91 (100), 65 (15).

3-Phenylpropanol: from 3-phenylpropanoic acid (18.4 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.25-7.10 (m, 5H, aromatic hydrogens), 3.60 (t, 2H, *J* = 6.4 Hz, $PhCH_2CH_2CH_2OH$), 2.65 (t, 2H, *J* = 7.2 Hz, $PhCH_2CH_2CH_2OH$), 1.83 (m, 2H, $PhCH_2CH_2CH_2OH$). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 141.5, 128.4, 128.3, 125.9, 62.5, 34.4, 32.1. GC/MS: t_R = 12.38 min, *m/z* 136 (M^+, 20), 117 (100), 91 (95).

2-Phenoxyethanol: from phenoxyacetic acid (25.9 mg, 94%) and from methyl phenoxyacetate (26.4 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.28 (m, 2H, aromatic hydrogens), 6.93 (3H, m, aromatic hydrogens), 4.08 (m, 2H, $PhOCH_2CH_2OH$), 3.94 (m, 2H, $PhOCH_2CH_2OH$). ^{13}C NMR (100

MHz, $CDCl_3$, TMS): δ = 158.6, 129.4, 121.0, 114.6, 69.2, 61.2. GC/MS: t_R = 11.82 min, *m/z* 138 (M^+, 27), 94 (100), 77 (32).

Furan-2-ylmethanol: from 2-furoic acid (35.3 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ =7.38 (m, 1H, heteromatic hydrogen), 6.32 (m, 1H, heteroaromatic hydrogen), 6.27 (m, 1H, heteroaromatic hydrogen), 4.59 (s, 2H, CH_2OH). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 153.6, 142.5, 110.5, 107.8, 57.0. GC/MS: t_R = 3.92 min, *m/z* 98 (M^+, 100), 81 (60), 63 (60).

2-(Thiophen-2-yl)ethanol: from 2-(thiophen-2-yl)acetic acid (3.7 mg, 72%) and from methyl 2-(thiophen-2-yl)acetate (24.6 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.20 (m, 1H, heteroaromatic hydrogen), 6.99 (m, 1H, heteroaromatic hydrogen), 6.90 (m, 1H, heteroaromatic hydrogen), 3.85 (t, 2H, *J* = 6.2 Hz, CH_2OH), 3.02 (t, 2H, *J* = 6.2 Hz, CH_2CH_2OH). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 140.5, 127.0, 125.8, 124.0, 63.4, 33.3. GC/MS: t_R = 9.47 min, *m/z* 128 (M^+, 30), 110 (5), 97 (100).

(E,E)-2,4-Hexadien-1-ol: from (*E*,*E*)-2,4-hexadienoic acid (2.9 mg, 74%) and from methyl (*E*,*E*)-2,4-hexadienoate (4.51 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.15 (dd, 1H, *J* = 15.0, 10.5 Hz, *CH*=C), 5.95 (dd, 1H, *J* = 15.0, 10.5 Hz, *CH*=C), 5.59 (m, 2H, 2*CH*=C), 4.00 (d, 2H, *J* = 6.0 Hz, CH_2OH), 1.68 (d, 2H, *J* = 6.2 Hz, CH_3C=). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 130.7, 129.5, 128.7, 125.6, 66.5, 17.4. GC/MS: t_R = 5.04 min, *m/z* 98 (M^+, 62), 83 (78), 55 (100).

1-Octanol: from methyl octanoate (9.10 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 3.63 (t, 2H, *J* = 6.6 Hz, CH_2OH), 1.56 (t, 2H, *J* = 6.5 Hz, CH_2), 1.40-1.20 (m, 10H, 5 CH_2), 0.88 (t, 3H, *J* = 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 63.1, 32.8, 31.8, 29.4, 29.2, 25.8, 22.6, 14.0. GC/MS: t_R = 8.36 min, *m/z* 112 (M^+–18, 5), 84 (53), 70 (70), 56 (100).

3. Results and discussion

Filamentous fungi are a huge group of organisms with great biodiversity. They have heterogeneous, complex and a-specific enzymatic patterns, which make them potentially valuable reagents for

biocatalysis. They are able to grow readily using simple carbon sources and with respect to many bacteria they can work at room temperature and pressure. *S. racemosum* belongs to Zygomycota phylum and particularly the strain MUT 2770 was isolated from air. Since it is not a human pathogen, there are no drawbacks to its use both in laboratory and in industrial plants.

We carried out a preliminary investigation of the substrate scope of *S. racemosum* reduction by using unsubstituted aromatic and aliphatic carboxylic acids, in order to define the basic structural scaffold of the substrate that could be accepted and transformed by the fungus. The corresponding methyl and ethyl esters were also employed as starting materials, in order to verify the possibility to slowly generate the carboxylic acid in the reaction medium by enzymatic hydrolysis.

The reactions were performed by addition of the starting compound (generally 5 mM final concentration in the reaction medium) to a pre-grown culture of *S. racemosum* in MEA medium, and incubated at 25°C. The conversion values determined by GC/MS analysis and the corresponding isolation yields are reported in Table 1. Benzoic acid, 2-(thiophen-2-yl)acetic acid, and 2,4-hexadienoic acid were not reduced when the starting substrate concentration (c_0) was 5 mM, whereas their conversion was nearly complete when a lower substrate loading was employed (c_0 = 1 mM). The reaction occurred on aromatic, heteroaromatic and aliphatic derivatives, either acids or methyl esters, whereas ethyl esters were not transformed. Remarkably, the presence of an electron-rich heteroaromatic ring does not prevent the reduction.

Table 1. Bioreduction of unsubstituted aromatic and aliphatic carboxylic acids and esters.[a]

Substrate	Product	c_0 (mM)[b]	Conversion (%)[c]	Isolation yield (%)[d]
PhCOOH	$PhCH_2OH$	5	0	–
PhCOOH	$PhCH_2OH$	1	>99	89
PhCOOMe	$PhCH_2OH$	5	>99	92
PhCOOEt	–	5	0	
$PhCH_2COOH$	$PhCH_2CH_2OH$	5	44	37
$PhCH_2COOMe$	$PhCH_2CH_2OH$	5	32 [47][e]	26
$PhCH_2COOEt$	–	5	0	
$PhCH_2CH_2COOH$	$PhCH_2CH_2CH_2OH$	5	73	68
$PhCH_2CH_2COOMe$	–	5	0	
$PhCH_2CH_2COOEt$	–	5	0	

$PhOCH_2COOH$	$PhOCH_2CH_2OH$	5	>99	94
$PhOCH_2COOMe$	$PhOCH_2CH_2OH$	5	>99	96
2-Furoic acid	2-Furfurol	5	>99	90
Methyl 2-furoate	2-Furfurol	5	8	
2-(Thiophen-2-yl)acetic acid	2-(Thiophen-2-yl)ethanol	5	0	–
2-(Thiophen-2-yl)acetic acid	2-(Thiophen-2-yl)ethanol	1	89	72
Methyl 2-(thiophen-2-yl)acetate	2-(Thiophen-2-yl)ethanol	5	>99	96
Octanoic acid	–	5	0	
Methyl octanoate	1-Octanol	5	45 [26][e]	35
(*E,E*)-2,4-Hexadienoic acid	(*E,E*)-2,4-Hexadien-1-ol	5	0	–
(*E,E*)-2,4-Hexadienoic acid	(*E,E*)-2,4-Hexadien-1-ol	1	>99	74
(*E,E*)-Methyl 2,4-hexadienoate	(*E,E*)-2,4-Hexadien-1-ol	5	32	23

[a] All experiments were performed as triplicates, deviations were always below 5% (see Experimental section for reaction conditions). [b] Starting substrate concentration. [c] Percentage yields of the primary alcohol, determined by GC analysis of the crude mixture after 72 h reaction time (no degradation or side-product formation was observed). [d] When conversion was not complete, purification by treatment with sat. $NaHCO_3$ solution or column chromatography was performed. [e] The percentage of the carboxylic acid obtained by ester hydrolysis is reported in square brackets.

After the preliminary screening, the effects on the bioreduction due to the substituents of the aromatic ring were investigated. The data of the biotransformations of substituted benzoic and phenylacetic acids and of the corresponding esters are reported in Figures 1 and 2, revealing quite a broad tolerance towards non-natural substrates. The methoxy group seems to be the substituent of the molecular skeleton best tolerated by the fungus. The three regioisomeric toluic acids and the corresponding methyl esters, and the three nitrobenzoic acids and methyl esters were recovered unreacted (data not shown in Figure 1). On the contrary, the methyl and nitro groups did not have a negative effect on the reduction of phenylacetic acid derivatives. For this latter class, the chlorine atom either in *ortho, meta* or *para* position (data not shown in Figure 2) prevented the reaction, whereas this inhibitory effect was decidedly of a lesser extent in the benzoic acid series.

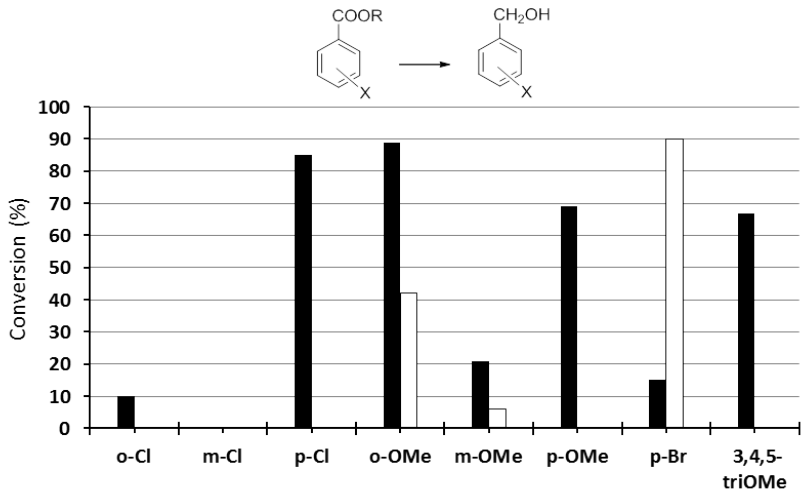


Figure 1: Percentage yields of the primary alcohols obtained by bioreduction of substituted benzoic acids (black bars) and methyl esters (white bars), determined by GC analysis of the crude mixture after 72 h reaction time.

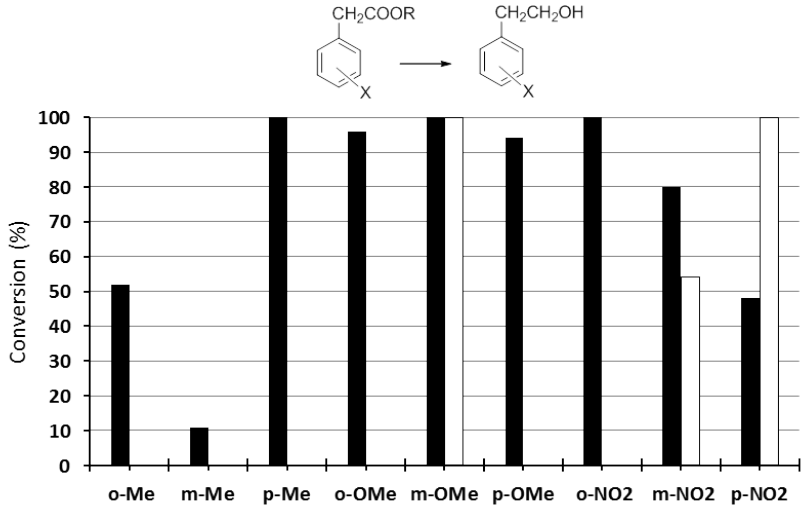


Figure 2: Percentage yields of the primary alcohols obtained by bioreduction of substituted phenylacetic acids (black bars) and methyl esters (white bars), determined by GC analysis of the crude mixture after 72 h reaction time.

The time course of the reaction (Figure 3a-b) was investigated for phenoxyacetic acid and its methyl ester, which were both completely reduced to the corresponding primary alcohol in 72 h reaction time.

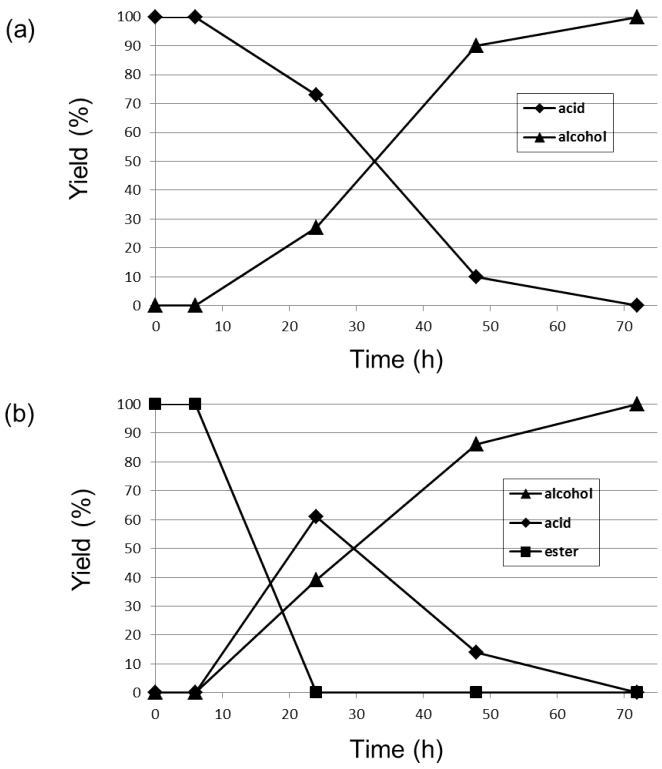


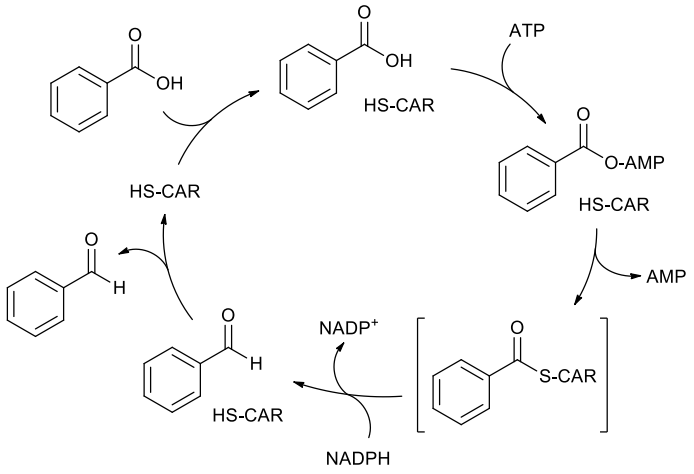
Figure 3: Time course of *S. racemosum*-catalysed reduction of phenoxyacetic acid (a) and methyl phenoxyacetate (b).

These data show that the hydrolysis of the ester occurs in the reaction medium to generate *in situ* the carboxylic acid, which probably diffuses across the cell membrane, and is then transformed by reduction.

The results collected in Table 1 and in Figures 1 and 2 highlight that for most acid/ester pairs only the carboxylic acid was transformed. In a few cases, both the acid and the methyl ester were converted into primary alcohols. Benzoic acid and some other carboxylic acids were recovered unreacted when c_0 = 5 mM was employed, whereas the corresponding methyl esters were reduced either totally or partially. For the latter derivatives, the slow release of the acid by hydrolysis in the fermentation medium likely allowed it to be reduced before its concentration could reach the critical value possibly toxic or inhibitory for the fungus. To support this hypothesis, the bioreduction of benzoic acid was investigated by decreasing the substrate starting concentration. With c_0 = 2.5 mM, 3% of benzaldehyde was observed after 24 h, and 97% of unreacted acid. The use of c_0 = 1 mM gave 28%

of benzaldehyde, 63% of benzyl alcohol and 9% of benzoic acid at 2 h reaction time, and complete conversion into benzyl alcohol after 4 h.

The detection of benzaldehyde as an intermediate of benzoic acid reduction is in agreement with the presence a CAR, whose accepted reduction mechanism is reported in Scheme 1.[14]



Scheme 1: Catalytic cycle for the reduction of benzoic acid to benzaldehyde by CAR isolated from *Nocardia* sp. NRRL 5646. HS-CAR represents phosphopantetheinylated CAR with the free terminal thiol group (see ref. 14).

The adenylation of benzoic acid occurs at the *N*-terminal domain of the enzyme by reaction with ATP, yielding the activated intermediate benzoyl-AMP and a pyrophosphoric acid unit. The nucleophilic attack of the phosphopantetheine thiol on benzoyl-AMP releases AMP and gives a covalently bound thioester. The latter is then reduced by NADPH at the *C*-terminal domain, releasing benzaldehyde, $NADP^+$ and the free HS-CAR ready for another reduction cycle.

In the investigations led on the reaction mechanism of CAR from *Neurospora crassa,*[6c] the formation of the activated intermediate had been described to be inhibited by the presence of hydroxylamine. The inhibition of the exchange reaction of benzoic acid with ATP by hydroxylamine was confirmed also in the case of *S. racemosum*: benzoic acid was not reduced when the reaction was performed with 1 mM substrate concentration in the presence of 1 mM hydroxylamine.

4. Conclusions

The *S. racemosum*-catalysed reductions of carboxylic acids, either employed as starting compounds or produced from esters by enzymatic hydrolysis, shows relevant advantages over known chemical methods. For example, the reduction of phenoxyacetic acid is described in the literature according to the following procedures: (i) with stoichiometric amounts of either titanium(IV) chloride and sodium borohydride (14 h) [15] or diisopropoxytitanium(III) tetrahydroborate (4 h) [16] at ambient temperature; (ii) with lithium aluminum hydride in refluxing THF [17]; (iii) with copper(II) triflate (16 h) at 80°C [18]; (iv) by reaction of the corresponding thiol ester with tetrabutylammonium borohydride (3 h) in refluxing chloroform [19]; (v) by reduction of the 6-nitro-1-(2-nitrophenylsulfonyloxy)benzotriazole derivative with sodium borohydride.[20] In the case of methyl benzoate, besides traditional hydride reductions, procedures based on the use of molecular hydrogen and ruthenium catalysts have been reported, most commonly at 100°C and 50 bar.[21]

The biotrasformation with *S. racemosum* whole cells system has all those characteristics of diminished environmental burden, reduced costs, and increased safety that are necessary to increment the sustainability of manufacturing processes.[22] It does not require a controlled atmosphere: neither oxygen has to be excluded, nor hydrogen has to be supplied. The reaction occurs in water medium with a minimum amount of organic solvents, with neither by-product formation, nor energy consumption for heating systems. Non-hazardous materials are employed under safe reaction conditions with reduced risk of exposure, explosions and fires, and without production of toxic waste. The reaction is easily performed on a semi-preparative scale (*ca.* 30 mg substrate), and the work-up for product recovery requires only a simple extraction procedure: neither any particular quenching, nor the filtration of metal salts as byproducts is needed.

Work has to be done to increase the reaction throughput, by optimising the fermentation conditions, and eventually isolating the enzymes responsible for the transformation. The chemoselectivity of the

biocatalysed procedure has to be evaluated on dicarboxylic acid derivatives and on substrates showing other functional groups, in order to define the synthetic potential of the reduction.

This kind of reaction enriches the enzymatic toolbox [23] which is made available to organic synthetic chemists, and which is still very scarce in the supply of biocatalysts for the reduction of carboxylic acids into primary alcohols. It can be considered for the development of green processes [24] for the biotechnological conversion of fatty acids, recovered from biomasses, into fatty alcohols, which are chemical commodities currently employed on a large scale for the production of fragrances, emollients, detergents, and food additives. *S. racemosum*-catalysed reduction can be also employed for the preparation of natural flavors and fragrances, because it satisfies the requisites established by European regulations for the natural labeling of the corresponding products.

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